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Mediator kinase disruption in *MED12*-mutant uterine fibroids from Hispanic women of South Texas

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Context: Mutations in the gene encoding Mediator complex subunit MED12 are dominant drivers of uterine fibroids (UFs) in women of diverse racial and ethnic origins. Previously, we showed that UF-linked mutations in MED12 disrupt its ability to activate Cyclin C-CDK8/19 in Mediator. However, validation of Mediator kinase disruption in the clinically relevant setting of MED12-mutant UFs is currently lacking.

Objective: The objective of this study was two-fold. First, to extend the ethnic distribution profile of MED12 mutations by establishing their frequency in UFs from Hispanic women of South Texas. Second, to examine the impact of MED12 mutations on Mediator kinase activity in patient-derived UFs.

Methods: We screened 219 UFs from 76 women, including 170 tumors from 57 Hispanic patients, for MED12 exon 2 mutations, and further examined CDK8/19 activity in Mediator complexes immunoprecipitated from MED12 mutation-negative and MED12 mutation-positive UFs.

Results: MED12 exon 2 mutations in UFs from Hispanic women are somatic in nature, predominantly monoallelic, and occur at high frequency (54.1%). We identified a minimal Cyclin C-CDK8 activation domain on MED12 spanning amino acids 15-80 that includes all recorded UF-linked mutations in MED12, suggesting that disruption of Mediator kinase activity is a principal biochemical defect arising from these pathogenic alterations. Analysis of Mediator complexes recovered from patient UFs confirmed this, revealing that Mediator kinase activity is selectively impaired in MED12-mutant UFs.

Conclusions: MED12 mutations are important drivers of UF formation in Hispanic women of South Texas. MED12 mutations disrupt Mediator kinase activity, implicating altered CDK8/19 function in UF pathogenesis.

Precis

MED12 exon 2 mutations were found at high frequency (54.1%) in UFs from Hispanic women of South Texas, leading to selective disruption of Mediator kinase activity in *MED12* mutation positive tumors.

Introduction

Uterine leiomyomas (uterine fibroids; UFs) are benign monoclonal neoplasms of the myometrium (MM) and represent the most common gynecological tumors in women worldwide (1,2). Tumors are estimated to occur in ~77% of women overall and are clinically manifest in ~25% by age 45 (1,2). Although benign, these tumors are nonetheless associated with significant morbidity; they are the primary indication for hysterectomy, and a major source of gynecologic and reproductive dysfunction, ranging from profuse menstrual bleeding and pelvic pain to infertility, recurrent miscarriage, and pre-term labor (1,2). Accordingly, the US annual health care costs associated with UFs have been estimated at ~$34 billion (3). Uterine fibroids thus represent a significant public health and financial burden.

Current treatment options for UFs are primarily surgical or radiological and range from hysterectomy or myomectomy to minimally invasive options, including uterine artery embolization (UAE) and magnetic resonance-guided focused ultrasound (MRgFUS) (4). However, the deleterious impact of these procedures on reproductive function is either clear (hysterectomy) or controversial (UAE, MRgFUS), rendering such options unsuitable for women who wish to retain future fertility (5). Likewise, hormonal therapies designed to blunt the stimulatory effects of estrogen or progesterone on fibroid growth are currently contraindicated in women actively pursuing a pregnancy, and are otherwise approved only for short-term use due to long-term safety concerns (6,7). Accordingly, no long-term noninvasive treatment option currently exists for UFs, and deeper mechanistic insight concerning tumor etiology will be key to develop newer targeted therapies.

In this regard, the prevailing model for UF pathogenesis invokes the genetic transformation of a single MM stem cell (MM SC) into a tumor-initiating cell (UF SC) that

seeds and sustains clonal tumor growth, characterized by an increase in cell size and number, as well as abundant extracellular matrix production, under the influence of endocrine, autocrine, and paracrine growth factor and hormone receptor signaling (8-10). Recent advanced genomic technologies, including high-throughput sequencing methodologies, have identified recurrent and mutually exclusive genetic alterations (i.e., drivers) thought dominantly responsible for cell transformation. Among these, somatic mutations in the *Xq13* gene encoding the RNA polymerase II (Pol II) transcriptional Mediator subunit MED12 are by far the most prevalent, occurring in 45-80% of UFs in various studies (11,12). Notably, *MED12* is recurrently mutated at high frequency in UFs from women of diverse racial and ethnic origins, including those of North American, European, African, Asian, and Middle Eastern descent, implicating *MED12* as a dominant universal driver of UFs (13-22). A proportionally smaller fraction of tumors are thought to arise from genetic alterations leading to overexpression of HMGA2 (~20%), disruption of COL4A5-COL4A6 locus (~3%), biallelic loss of fumarate hydratase (FH; ~2%), or unknown molecular genetic processes (12,23). Additionally, recurrent deletions and rearrangements involving chromosomes 6p21, 7q22, 22q, and 1p have been observed in UFs; however, these lesions generally co-occur with other genetic alterations, suggesting that they may represent secondary driver events restricted to a subpopulation of tumor cells (17,24-26). Altogether, the identification of different prospective driver mutations in UFs suggests the existence of distinct molecular subtypes with possibly unique pathways to tumorigenesis.

The revelation that *MED12* is recurrently mutated at high frequency in UFs implicates dysregulation of RNA polymerase II (Pol II)-dependent gene expression in fibrotic transformation. Mediator is a conserved multiprotein interface between gene-specific transcription factors and Pol II (27). In this capacity, Mediator channels regulatory signals from activator and repressor proteins to affect changes in gene expression programs that

control diverse physiological processes, including cell growth and homeostasis, development, and differentiation. Structurally, Mediator is assembled from a set of 26 core subunits into three distinct modules termed “head”, “middle”, and “tail” that bind tightly to Pol II in the so-called holo-enzyme (27). MED12, MED13, CycC, and CDK8 (or its paralog CDK19) comprise a four-subunit “kinase” module that variably associates with core Mediator (27). The kinase module has been implicated in activation as well as repression of transcription through mechanisms both dependent and independent of its resident CDK8/CDK19 kinase activity. Mediator kinase-dependent gene regulation has been attributed to CDK8/19-targeted phosphorylation events that impact transcription factor half-life, Pol II activity, and chromatin chemistry and functional status (27,28). Notably, the kinase module is a major ingress of signal transduction through Mediator, and MED12-dependent CDK8 activation is required for nuclear transduction of signals instigated by multiple oncogenic pathways with which MED12 is biochemically and genetically linked (27). Furthermore, MED12 is a target of oncogenic mutation in colon, prostate, and renal cell carcinomas (29-31). However, these mutations occur predominantly in the MED12 C-terminus and thus lie distant from UF-linked mutations that cluster in its N-terminus, suggesting possible distinct etiological mechanisms (32).

Regarding UF-linked mutations in *MED12*, all lesions heretofore recorded impact exons 1 and 2 and most are missense, with a smaller proportion corresponding to in frame deletions and insertions (16,23,27). UF-linked *MED12* exon 2 mutations are far more frequent than those occurring in exon 1, with latter accounting for ~6% of pathogenic alterations reported in uterine fibroids (23). Although missense mutations in exon 2 are distributed throughout the coding sequence, most are clustered in codons 36, 43, and 44, suggesting an important function for their corresponding and highly conserved amino acid residues. Along with their high frequency occurrence, two additional genetic

findings suggest that *MED12* mutations are drivers of fibrotic transformation. First, predominant monoallelic expression of mutant *MED12* has been observed in UF tumors, indicative of a pathogenic requirement for a functionally altered *MED12* allele (16,23,27). Second, directed expression of a *MED12* mutant transgene (c. 131G>A; p.G44D) in the uterine compartment of mice is sufficient to induce UF formation, providing direct genetic proof of disease causality (33). Nonetheless, the impact of UF-linked mutations on MED12 function and the molecular basis for their tumorigenic potential remain to be clarified.

In this regard, we previously reported that UF-linked exon 1 and 2 mutations in *MED12* lead to disruption of Mediator-associated CDK activity, with significant implications for global dysregulation of gene expression programs. Mechanistically, we showed that these UF-linked mutations in MED12 disrupt its ability to bind directly to CycC, an interaction necessary for MED12-mediated activation of CycC-dependent CDK8/19 within Mediator (23,34,35). These findings identified for the first time a common molecular defect associated with UF-linked mutations in MED12 and further implicate aberrant CDK8/19 activity in UF pathogenesis. Nonetheless, direct validation of Mediator kinase disruption in the clinically relevant setting of *MED12*-mutant uterine fibroid tumors has only very recently been reported from a restricted set of Caucasian (Finnish patients) (34). Therefore, the objective of this study was two-fold; first, to establish the frequency of *MED12* mutations in UFs from Hispanic women of south Texas in an effort to further catalog *MED12* driver alterations in diverse ethnic populations, and second, to examine the impact of tumorigenic *MED12* mutations on Mediator kinase activity in clinically relevant patient fibroids. To this end, we screened a total of 219 fibroid tumors from 76 women, including a large subset from Hispanic patients, for *MED12* exon 2 mutations, and further examined kinase activity within Mediator complexes recovered

from *MED12* mutation-negative and *MED12* mutation-positive UFs as well as adjacent normal myometrium. We found that *MED12* exon 2 mutations occur at high frequency (54.1%) in Hispanic patients, suggesting that *MED12* mutations are important drivers of UF formation in this ethnic population. Moreover, we document that Mediator kinase activity is indeed selectively and severely impaired in *MED12*-mutant UFs. Together, these findings confirm in a clinically relevant setting that UF-linked mutations in *MED12* disrupt Mediator-associated CDK activity and provide additional evidence to implicate altered CDK8/19 activity in the pathogenesis of *MED12*-mutant uterine fibroids.

Materials and Methods

Patient Samples

This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio. Uterine fibroid and myometrium samples were collected as fresh frozen tissues from informed consent patients undergoing hysterectomy. Sample histology was reviewed by a board certified gynecologic pathologist. In total, 219 uterine fibroid and 28 myometrium samples from 76 patients, including 57 Hispanic women, 9 African American women, 8 Caucasian women, 1 Iranian woman, and 1 Chinese woman were analyzed. Patient age ranged from 28-61 years with a mean of 42.4 years.

Mutation Analysis

Genomic DNA from UF tumors and corresponding myometrial samples was extracted from 100 mg of fresh tissues using tissue lysis buffer (10mM Tris pH 7.5, 10mM EDTA pH 8.0, 10mM NaCl, 0.5% sodium sarcosyl) with proteinase K followed by ethanol precipitation. *MED12* exon 2 mutations were screened by polymerase chain reaction (PCR) direct sequencing. The primer sequences in the 5' to 3' direction were AAGTGAACGTAAGGGCCCAG (forward) and

AATGGCACTCTGGGATCGTG (reverse). The PCR products were purified with Gel Extraction Kit (QIAGEN, Valencia, CA, USA) prior to Sanger sequencing (GENEWIZ, South Plainfield, NJ, USA). The sequences were analyzed manually for the *MED12* gene exon 2 somatic mutations.

RNA extraction and RT-PCR

Tissue samples (100 mg) were treated with TRI Reagent (Life Technologies) as recommended by the manufacturer and RNA was extracted using Direct-zol RNA mini prep kit (Zymo Research). The RNA concentration and purity were determined by spectrophotometry. 1ug of RNA was converted to cDNA using ImProm-II Reverse Transcription System (Promega) according to the manufacturer's instructions.

cDNA sequencing

MED12 exon 2 cDNAs from uterine fibroid tissues were sequenced to verify that the mutated allele was actively expressed in each tumor. The primer sequences in the 5' to 3' direction are GGCTTCCCTCGGTAGTTTCC (forward) and TGCTGCATAGTAGGCACAGG (reverse) covering all the observed mutations. PCR products were gel purified using the QIAGEN PCR purification Kit (QIAGEN, Valencia, CA, USA) prior to Sanger sequencing (GENEWIZ, South Plainfield, NJ, USA).

Glutathione S-Transferase (GST) Pull-down and Kinase assays

GST-MED12 derivatives, including GST-MED12 (1-100) and its N-terminal (10-100, 15-100, 20-100) and C-terminal (1-60, 1-72, 1-80) truncation forms were purified from *E.coli* lysates using Glutathione Sepharose 4B for 1 hour at 4ºC. Beads were washed 4 times with Lysis250 (50mM Tris pH 7.5, 250mM NaCl, 5mM EDTA) and insect cell lysates containing baculovirus-expressed recombinant human $CycCH_6$-CDK8-FLAG proteins were incubated with immobilized

GST-MED12 derivatives for 1 hour at 4ºC. Complexes were washed 4 times in Lysis250 and either eluted in Laemmli sample buffer and resolved by SDS-10%-PAGE for western blot analysis, or incubated with kinase reaction buffer (25 mM Tris pH 7.5, 20 mM $MgCl_2$), 2.5 mCi [γ-^{32}P] ATP and 2 ug of purified GST or GST-3xCTD substrate bearing 3 tandem copies of a consensus heptapeptide sequence from the RNA Pol II large subunit carboxyl-terminal domain. Kinase reactions were incubated for 30 minutes at 30ºC, eluted in Laemmli sample buffer, processed by SDS-12% PAGE, and stained with Coomassie stain and visualized by phosphorimager analysis. ^{32}P-labeled GST-CTD was quantified using ImageQuant software.

Immunoprecipitation from human tissue samples

Fresh frozen myometrium and uterine fibroid tissues were homogenized at 4 ºC in protein lysis buffer (40mM Tris pH 7.4, 500mM NaCl, 0.5% Sodium-deoxycholic acid, 1% Triton X-100, and 1mM EDTA). Tissue homogenates were pre-cleared by incubation with protein A-agarose. Pre-cleared lysates were then incubated with anti-MED12 antibody covalently to protein A-agarose (Millipore Corp). As a negative control, tissue lysates were incubated with normal rabbit IgG-agarose conjugate (Santa Cruz Biotechnology, Inc). Immunoprecipitations were performed for 3 hour at 4 ºC. The beads were washed three times with 400 ul of wash buffer (40mM Tris pH 7.4, 500mM NaCl, 1mM EDTA). Immunoprecipitaes were either eluted in Laemmli sample buffer and either processed by SDS-10% PAGE for western blot analysis, or incubated with kinase reaction buffer (25 mM Tris pH 7.5, 20 mM $MgCl_2$), 2.5 mCi [γ-^{32}P] ATP and 2 ug of purified GST or GST-3xCTD. ^{32}P-labeled GST-3xCTD was resolved by SDS-12% PAGE, stained with Coomassie stain and visualized by Phosphoimager analysis. The ^{32}P-labeled GST-3xCTD was quantitated using ImageQuant software, and levels of phosphorylation from MED12-mutant immunoprecipates were relatively compared to those from MED12 WT immunoprecipitates.

Results

MED12 is frequently mutated in UFs from Hispanic women of South Texas

In total, we sequenced 219 UFs from 76 patients, including 170 tumors from 57 Hispanic women, for evidence of *MED12* exon 2 mutations (Tables 1 and 2; Supplementary Tables S1-S4). In addition, matched myometrial tissues available from 28 patients were also included for sequence analysis (Tables S3 and S4). Among sequenced UFs, 121 of 219 tumors total (55.3%), including 92 of 170 (54.1%) from Hispanic patients, harbored a mutation in *MED12* exon 2 (Fig. 1 and Table 2; Fig. S1 and Tables S3 and S4). Notably, the vast majority of these *MED12* mutations [104 of 121 total (85.9%); 79 of 92 from Hispanic patients (85.8%)] corresponded to missense mutations in codon 44 (Table 3). In addition, among the 121 total *MED12*-mutant UFs, 10 carried a missense mutation in codon 36, 1 carried a missense mutation in codon 68, and 6 carried in-frame exonic deletions that variously spanned 3-13 codons in length (Table 3). Among the 92 UFs from Hispanic patients, 8 harbored codon 36 mutations and 4 displayed in-frame exonic deletions. As expected, no mutations were found in adjacent myometrium, confirming the somatic nature of the UF mutations in *MED12* (Fig. 1; Fig. S1; Tables S3 and S4). All tumors examined carried only one *MED12* mutation, and all mutations were heterozygous in nature, with the mutant allele predominantly expressed. Thus, cDNA sequencing revealed that tumors harboring missense mutations and deletions internal to exon 2 expressed both mutated and wild-type alleles, with the former generally more abundant than the latter (Fig. 1). Consistent with prior published findings, a significant correlation was observed between *MED12* mutation status and fibroid tumor size, with tumors carrying the most frequent MED12 mutation (c.131G>A; p.G44D; 40/121 or 33% of total UFs) found to be statistically significantly smaller than those without MED12 mutations ($P < 0.01$). Interestingly, however, this relationship was lost when all *MED12* mutations were

considered, a distinction not observed in previous studies. Beyond tumor size, no correlations were observed between *MED12* mutation status and either UF number or location, nor were any relationships noted between *MED12* mutation status and patient age, BMI, or parity.

Identification of a minimal CycC-CDK8/19 binding and activation domain on MED12

Previously, we and others have shown that MED12 is an obligate activator of CycC-CDK8/19 in Mediator (34,35). Mechanistically, we showed that MED12 allosterically activates both CDK8 and CDK19 through a direct interaction between MED12 and a phylogenetically conserved surface groove on CycC (34,35). Importantly, we mapped the CycC-binding interface on MED12 to its N-terminal 100 amino acids [MED12 (1-100)] and further showed that UF-linked exon 1 and 2 mutations, all of which lie within MED12 (1-100), disrupt the ability of MED12 to bind CycC and thus activate CDK8/19 (34,35). To further delineate the CycC-binding (and thus CDK8/19 activation) domain on MED12, we used purified recombinant GST-MED12 (1-100) to generate a derivative series of N- and C-terminal MED12 truncation mutants (Fig. 2A), each of which was tested for its respective ability to bind and activate recombinant baculovirus-expressed CycC-CDK8. As expected GST-MED12 (1-100) exhibited robust CycC-CDK8 binding and activation function (Fig. 2B and C). Stepwise truncation of C-terminal residues from MED12 (1-100) revealed that deletion of more than 20 amino acids significantly impaired its ability to bind and activate CycC-CDK8. Thus, whereas GST-MED12 (1-80) bound and activated CycC-CDK8 comparably to GST-MED12 (1-00), GST-MED12 (1-72) exhibited little activity (Fig. 2B). In contrast to the stark reduction in CycC-CDK8 binding and stimulatory activity observed upon stepwise truncation of C-terminal residues, serial truncations from the N-terminus of MED12 (1-100) led instead to a gradual loss of function, eventually resulting in

significantly impaired CycC-CDK8 binding and stimulatory activity following deletion of the first 15 amino acids of MED12. Thus whereas GST-MED12 (15-100) retained ~80% of the CycC-CDK8 binding and stimulatory activity of GST-MED12 (1-100), GST-MED12 (20-100) exhibited only ~30% of such activity (Fig. 2C). Together, these analyses delimit the CycC-CDK8 binding and activation domain on MED12 to amino acids 15-80 that completely circumscribe the region on MED12 (amino acids 26-68) affected by UF-linked MED12 mutations (Fig. 2D).

Mediator kinase activity is selectively disrupted in *MED12* mutation-positive UFs

The observation that all UF-linked mutations in MED12 occur exclusively within its CycC-CDK8 binding and activation domain lends strong support for the notion that disruption of Mediator kinase activity is a primary molecular defect arising from these oncogenic alterations in MED12. In fact, our prior discovery that UF-linked exon 1 and 2 mutations in MED12 disrupt its CycC-CDK8/19 binding and activation functions directly supports this hypothesis (23,35). However, these prior findings arose from biochemical and cell biological studies using purified recombinant proteins or ectopically expressed MED12 WT and mutant derivatives in non-uterine cells. More recently, we validated these findings in the clinically relevant setting of *MED12* mutation positive UFs; however, these observations derived from analysis of UF tumors from a relatively restricted set of Caucasian (Finnish) patients (34). Therefore, to examine the functional impact of *MED12* mutations in UFs from a more diverse (Hispanic) patient pool, we comparatively assessed MED12-specific immunoprecipitates from *MED12* WT and mutant UFs for CDK8/19 kinase activity. For these experiments, UF samples from patients harboring MED12 WT or MED12 mutant (G44R, G44D, G44V) tumors were used for comparative analyses. Notably, all of the mutant MED12 proteins were expressed and co-precipitated Mediator subunits comparably to

WT MED12, indicating that UF-linked mutations in MED12 do not aberrantly affect its stable expression or incorporation into Mediator (Fig. 3A-C, top panels). Importantly, as predicted from our prior studies, CDK8/19 kinase activity was significantly impaired in mutant MED12/Mediator complexes compared to their WT counterparts (Fig 3A-C, bottom panels). These findings confirm that Mediator kinase activity is selectively disrupted in *MED12*-mutant uterine fibroid tumors.

Discussion

Herein, we show that *MED12* is recurrently mutated at high frequency (54.1%) in UFs from Hispanic women, leading to disruption of Mediator-associated kinase activity. This *MED12* mutation frequency is similar to reported frequencies in women of Korean (52.2%), Chinese (46.2%), Iranian (34.1%), and South African (50%) ancestry, but lower than that reported in Finnish (Caucasian) and North American (African American and Caucasian) women, where *MED12* mutation frequencies range from 60-85% in various studies (13-22). Whether these observed differences in the *MED12* mutation frequency reflect study bias (e.g., whole exome versus targeted sequencing, size of fibroids selected for analysis, etc.) or *bona fide* racial and ethnic disparity will require further analyses with expanded data sets. We note that the *MED12* mutation frequency reported herein may represent an underestimate of the actual number in the Hispanic population, since our sequencing analysis was restricted to exon 2, whereas exon 1 is also a target for pathogenic *MED12* mutations. Nonetheless, mutations in exon 1 account for ~6% of all those recorded in UFs, and therefore, any underestimate in the actual *MED12* mutation frequency reported herein is likely to be small (23). Altogether, our tumor analyses provide further confirmation that MED12 driver mutations are common in UFs from women of diverse racial and ethnic backgrounds, including Hispanic women.

Within Mediator, MED12 binds directly to CycC, and this interaction is essential for MED12-mediated activation of CDK8/19. In this study, we mapped the minimal CycC-binding and CDK8 activation domain on MED12 to amino acids 15-80 that completely encompass MED12 residues (amino acids 26-68) impacted by UF-linked mutations. Accordingly, the fact that no UF-linked mutations in MED12 lie outside of its biochemically defined CycC-CDK8 binding and activation domain argues strongly that Mediator kinase disruption is the principal biochemical defect arising from these oncogenic mutations. Herein, we validate this prediction in the pathologically relevant setting of patient-derived UFs. Thus, comparative analyses of Mediator complexes recovered from WT and mutant MED12-expressing UFs confirmed unequivocally that UF-linked MED12 mutations disrupt Mediator kinase activity, implicating CDK8/19 in UF pathogenesis.

The mechanistic basis by which Mediator kinase disruption contributes to UF formation remains to be established, but likely involves dysregulation of CDK8/19-dependent gene expression programs. Consistent with this notion, we previously found by comparative gene expression profiling that *MED12* WT and *MED12* mutant UFs stratify according to their unique gene expression signatures (23,36), suggesting that *MED12* mutant UFs constitute a distinct molecular subtype with a unique path to tumorigenesis. Furthermore, we note that Mediator kinase activity is known to regulate multiple signaling pathways linked to UF development, including the WNT/β-catenin, TGF-β, and estrogen receptor α (ERα) pathways, among others. In this regard, canonical WNT/β-catenin signaling is implicated in UF growth, and recent studies suggest its involvement as a paracrine effector of estrogen signaling in UF stem cells (37). Furthermore, *MED12*-mutant tumors support elevated levels of WNT4 expression (17). Notably, the Mediator kinase module has been linked directly to control of WNT/β-catenin signaling, first by our finding that MED12

is a direct transducer of WNT-activated β-catenin, and subsequently by the discovery that CDK8 promotes oncogenic WNT signaling by virtue of its dual role as a β-catenin coactivator and a suppressor of E2F1, a negative regulator of β-catenin (27). TGF-β is a key regulator of UF fibrosis and growth. TGF-β signaling stimulates smooth muscle cell proliferation and promotes fibroid formation through stimulation of ECM-promoting genes and inhibition of matrix-resorbing genes (38). Significantly, MED12 is an established suppressor of oncogenic TGF-β signaling, and CDK8 has been shown to instigate a phosphorylation-dependent SMAD action turnover switch that regulates the amplitude and duration of TGFβ-driven and SMAD-dependent transcriptional responses (39). Finally, ERα, as a principal mediator of estrogen action, is an important promoter of UF growth, and CDK8 was recently identified as a potent downstream mediator of transcriptional and mitogenic signaling by ERα (40). Thus, disruption of Mediator kinase activity as a consequence of pathogenic mutations in *MED12* could trigger dysregulated signal-dependent gene expression programs that contribute to UF formation. Nonetheless, CDK8 has been shown to phosphorylate a plethora of additional substrates with established or prospective roles in gene regulation, including DNA-binding transcription factors, components of the Pol II transcriptional apparatus, and diverse signaling molecules, including those involved in DNA damage response and repair (28). Further studies will be required to identify key substrates of Mediator kinases most relevant to UF pathogenesis.

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Figure Legends

Figure 1. Representative sequence chromatograms reveal *MED12* codon 44 mutation status in patient-derived UFs and myometrium. Examples of genomic DNA (**A, C**, **E**) and cDNA (**B**, **D**, **F**) sequencing traces in codon 44 mutated UF samples and a wild-type UF sample (**G**) is shown along with a genomic DNA sequencing trace from a myometrium sample (**H**). Codon 44 is highlighted by the horizontal bars below the traces. Mutated bases are indicated by arrows.

Figure 2. Identification of the minimal CycC-CDK8 binding and activation domain on MED12. (**A**) Schematic diagram of GST-MED12 (1-100) C-terminal and N-terminal truncation derivatives used in binding and activation assays. (**B** and **C**) Glutathione-sepharose-immobilized GST or GST-MED12 (1-00) and its C-terminal (**B)** and N-terminal (**C**) truncation derivatives as indicated were incubated with whole cell lysates from insect cells co-expressing baculovirus-produced human CycC-CDK8. Bound proteins were eluted with Laemmli sample buffer and processed by western blot (WB) using the indicated antibodies or incubated with [γ-^{32}P]-ATP and purified GST-CTD prior to resolution by SDS-PAGE and phosphorimager analyses (CTD-^{32}P). Coomassie blue stained gels show the levels of GST-MED12 derivatives (marked by bullets) and GST-CTD substrate (CTD) used in binding and kinase reactions, respectively. Molecular weight markers (kD) are indicated. Input (IN) corresponds to 10% of insect cell lysate used in IP reactions. ^{32}P-GST-CTD levels were quantified and expressed relative to the level obtained in the presence of GST-MED12 (1-100), which was assigned a value of 100%. Data represent the average +/- SEM of 3 independent experiments. Asterisks denote statistically significant differences versus MED12-GST (1-100)-stimulated kinase activity (Student's *t* test, *** $p < 0.001$: * $p < 0.01$). (**D**) Schematic diagram indicating

566 the experimentally defined minimal CycC-CDK8 binding and activation (bind/act) domain
567 relative to MED12 exon sequences. This region (amino acids 15-80) circumscribes all
568 recorded UF linked mutations in MED12.
569
570 **Figure 3.** Mediator kinase activity is selectively disrupted in MED12-mutant UF tumors. Whole
571 tissue lysates from patient-matched (**A**) or unmatched (**B** and **C**) UF tumor sets, including one
572 MED12 WT and one MED12 mutant UF tumor each, were subjected to IP with MED12-specific
573 antibodies or control IgG as indicated. Patient-matched samples in (**A**) correspond to UF
574 tumor 104Fa (MED12 WT) and UF tumor 104Fb (MED12 G44R). Unmatched samples in (**B**)
575 correspond to UF tumor 104Fa (MED12 WT) and UF tumor 102Fa (MED12 G44D). Unmatched
576 samples in (C) correspond to UF tumor 114Fb (MED12 WT) and UF tumor 128Fa (MED12 G44V).
577 MED12-specific IPs were resolved by SDS-10% PAGE and processed by WB analysis using the
578 indicated Mediator subunit-specific antibodies (top panels) or subjected to in vitro kinase
579 assay prior to resolution by SDS-PAGE and phosphorimager analyses (bottom panels). Input
580 corresponds to 10% of tissue lysates used in IPs. Molecular weight markers (kD) are indicated.
581 ^{32}P-GST-CTD levels were quantified and expressed relative those obtained in kinase reactions
582 with WT MED12/Mediator IPs which were assigned a value of 100%.

Figure 1

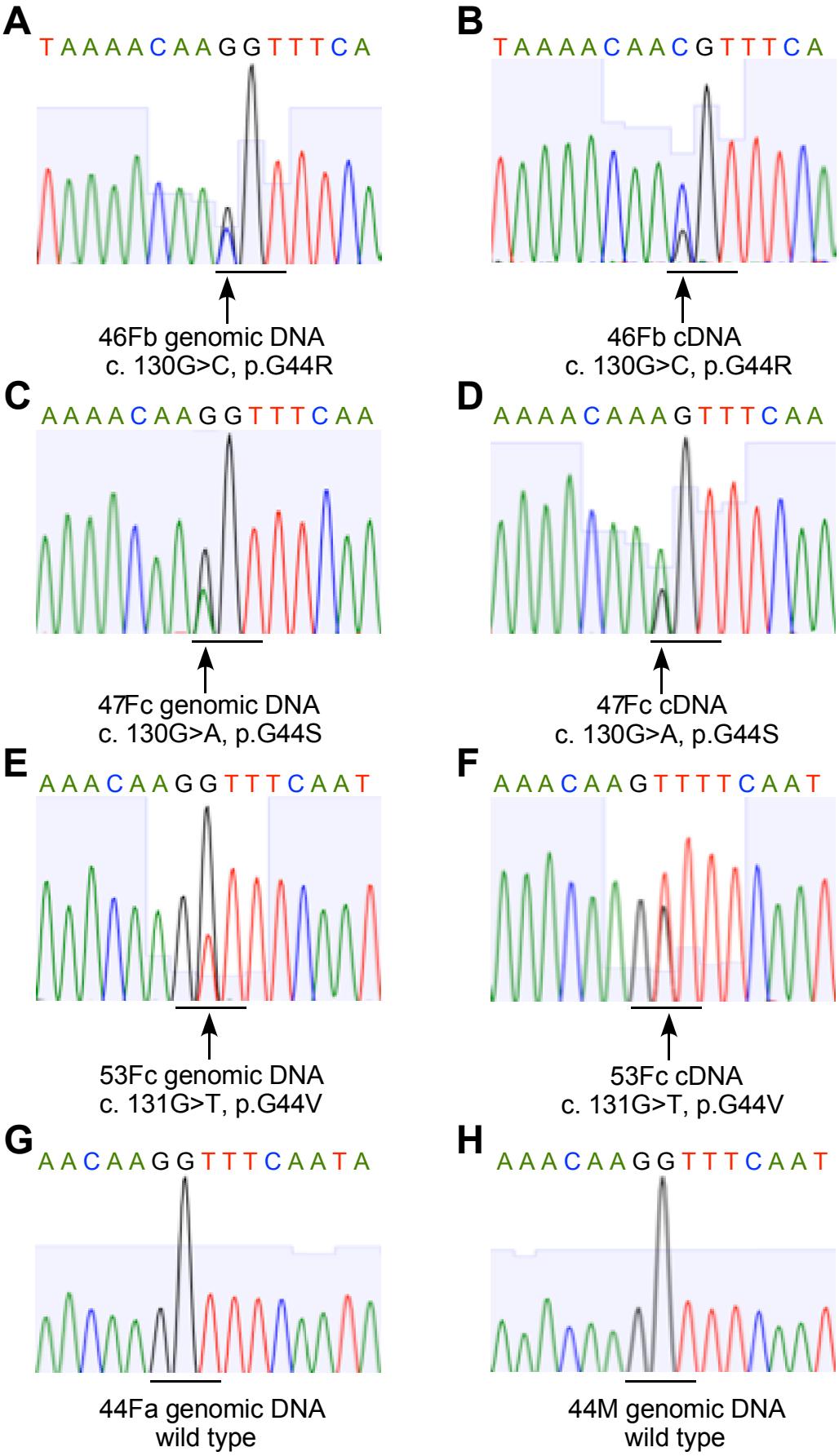

A
T A A A A C A A G G T T T C A
46Fb genomic DNA
c. 130G>C, p.G44R
B
T A A A A C A A C G T T T C A
46Fb cDNA
c. 130G>C, p.G44R
C
A A A A C A A G G T T T C A A
47Fc genomic DNA
c. 130G>A, p.G44S
D
A A A A C A A A G T T T C A A
47Fc cDNA
c. 130G>A, p.G44S
E
A A A C A A G G T T T C A A T
53Fc genomic DNA
c. 131G>T, p.G44V
F
A A A C A A G T T T T C A A T
53Fc cDNA
c. 131G>T, p.G44V
G
A A C A A G G T T T C A A T A
44Fa genomic DNA
wild type
H
A A A C A A G G T T T C A A T
44M genomic DNA
wild type

Figure 2

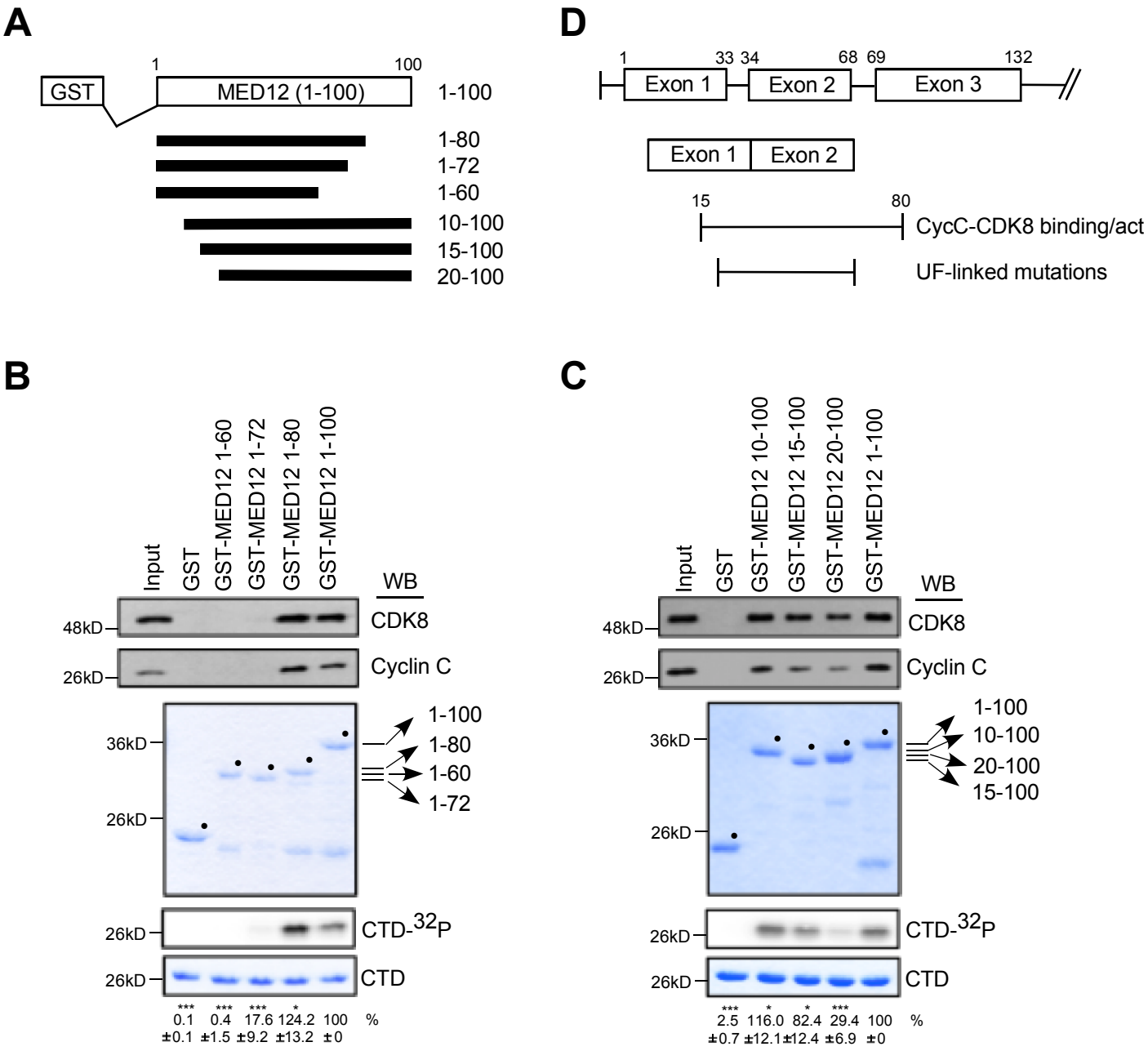
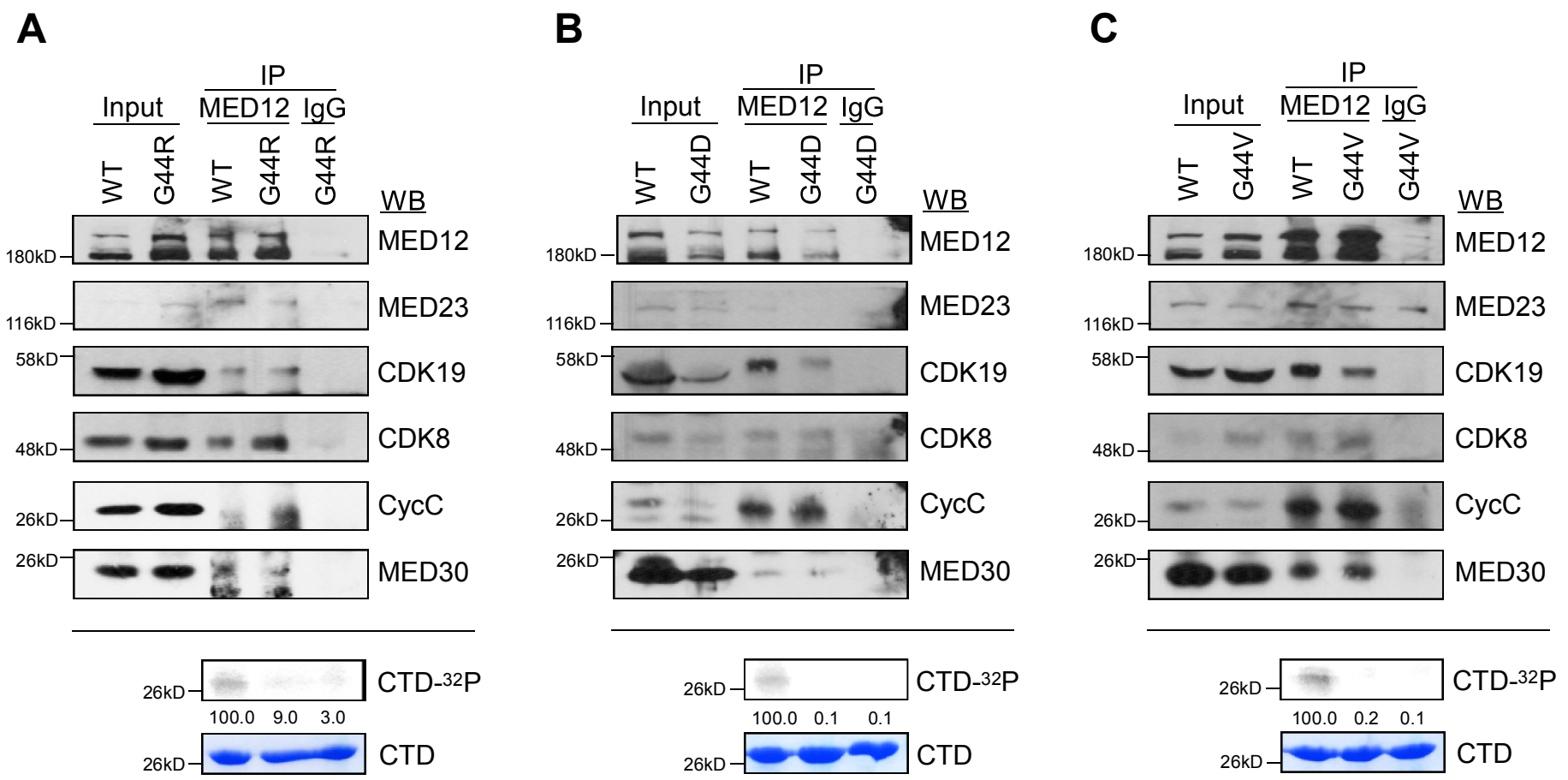

A
1
100
GST
MED12 (1-100)
1-100
1-80
1-72
1-60
10-100
15-100
20-100
D
1
33 34
68 69
132
Exon 1
Exon 2
Exon 3
Exon 1
Exon 2
15
80
CycC-CDK8 binding/act
UF-linked mutations
B
Input
GST
GST-MED12 1-60
GST-MED12 1-72
GST-MED12 1-80
GST-MED12 1-100
WB
48kD
CDK8
26kD
Cyclin C
36kD
26kD
1-100
1-80
1-60
1-72
26kD
CTD-32P
26kD
CTD
***
0.1
±0.1
***
0.4
±1.5
***
17.6
±9.2
*
124.2
±13.2
100
±0
%
C
Input
GST
GST-MED12 10-100
GST-MED12 15-100
GST-MED12 20-100
GST-MED12 1-100
WB
48kD
CDK8
26kD
Cyclin C
36kD
26kD
1-100
10-100
20-100
15-100
26kD
CTD-32P
26kD
CTD
***
2.5
±0.7
*
116.0
±12.1
*
82.4
±12.4
***
29.4
±6.9
100
±0
%

Figure 3


A
Input
IP
MED12
IgG
WT
G44R
WT
G44R
G44R
WB
180kD
MED12
116kD
MED23
58kD
CDK19
48kD
CDK8
26kD
CycC
26kD
MED30
26kD
CTD-32P
100.0 9.0 3.0
26kD
CTD
B
Input
IP
MED12
IgG
WT
G44D
WT
G44D
G44D
WB
180kD
MED12
116kD
MED23
58kD
CDK19
48kD
CDK8
26kD
CycC
26kD
MED30
26kD
CTD-32P
100.0 0.1 0.1
26kD
CTD
C
Input
IP
MED12
IgG
WT
G44V
WT
G44V
G44V
WB
180kD
MED12
116kD
MED23
58kD
CDK19
48kD
CDK8
26kD
CycC
26kD
MED30
26kD
CTD-32P
100.0 0.2 0.1
26kD
CTD

Supplemental Figure S1

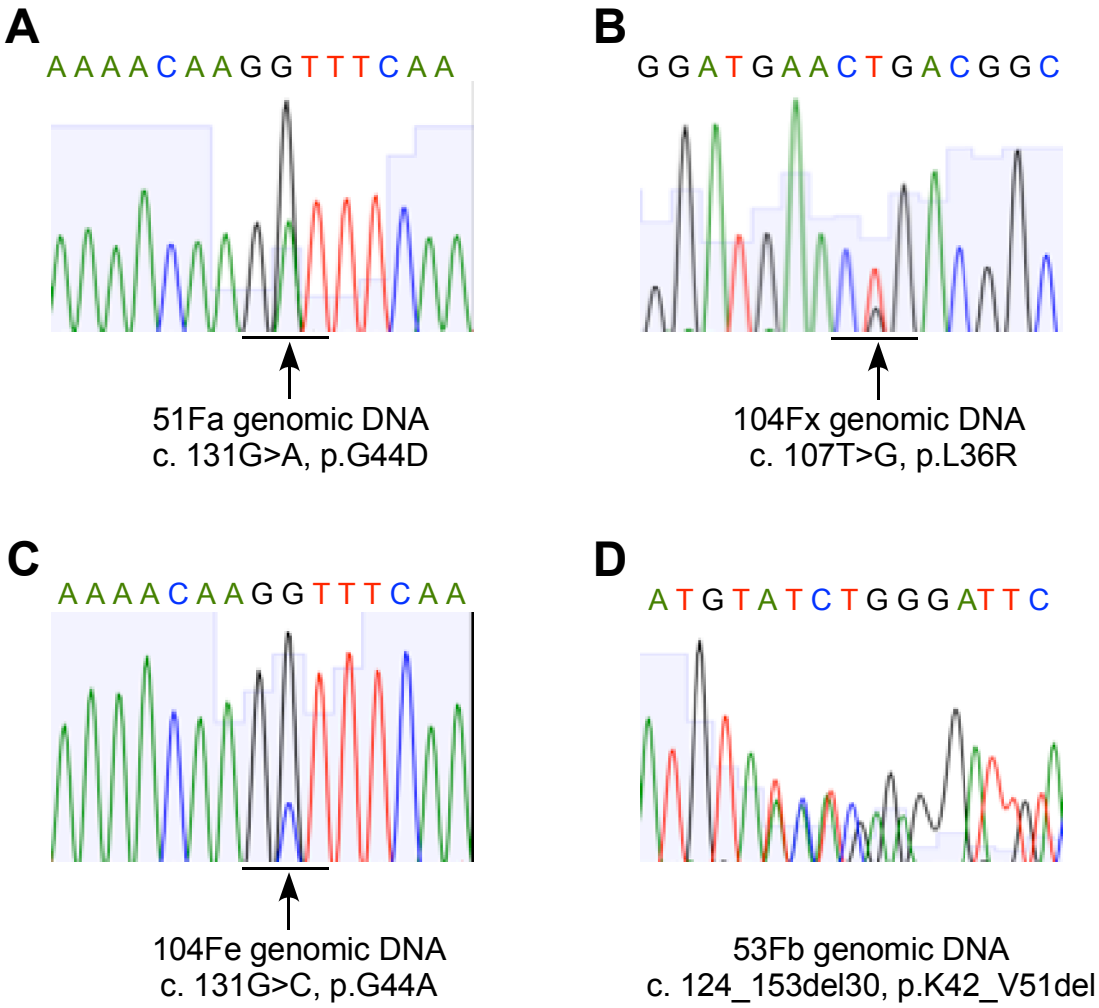


Table 2

Table 2. MED12 mutation frequency among UF patients

	# of Patients	# of Myometrium sequenced	# of Fibroids sequenced	# of MED12 Mut	Mutation (%)
Total	76	28	219	121	55.3%
Hispanic	57	21	170	92	54.1%
Other	19	7	49	29	59.2%

Table 3

Table 3. MED12 mutation type in UF tumors

Type	Location	Nucleotide change	Predicted protein change	Number of mutations out 121 (%)
Missense	Exon 2	c.131G>C	p.G44A	5 (4.1)
	Exon 2	c.130G>T	p.G44C	8 (6.6)
	Exon 2	c.131G>A	p.G44D	40 (33.1)
	Exon 2	c.130G>C	p.G44R	8 (6.6)
	Exon 2	c.130G>A	p.G44S	25 (20.7)
	Exon 2	c.131G>T	p.G44V	18 (14.9)
	Exon 2	c.107T>G	p.L36R	10 (8.3)
	Exon 2	c.204A>G	p.K68E	1 (0.8)
Deletion	Exon 2	c.117_131del15	p.L39P_G44del	1 (0.8)
	Exon 2	c.100-9_132del42insGG	p.D34_G44del	1 (0.8)
	Exon 2	c.100-2_138del41	p.D34_N46del	1 (0.8)
	Exon 2	c.139_153del15	p.N47_V51del	1 (0.8)
	Exon 2	c.124_153del30	p.K42_V51del	1 (0.8)
	Exon 2	c.100_144del45	p.D34_Q48del	1 (0.8)

Supplemental Table S1

Table S1. Summary of clinicopathological data for Hispanic UF patients

Individual	Ethnicity	Age	Solitary/Multiple fibroids	Uterine Fibroids	Diameter (cm)
7	Hispanic	37	Multiple	7b	8 cm
10	Hispanic	41	Multiple	10Fa	4 cm
				10Fb	3.5 cm
				10Fc	2.5 cm
11	Hispanic	56	Multiple	11Fa	4 cm
				11Fb	5 cm
				11Fc	NR
				11Fd	4 cm
12	Hispanic	43	Single	12F	8 cm
MRKH	Hispanic	29	Single	MRKH-M	NR
				MRKH-F	8 cm
14	Hispanic	44	Single	14Fa	< 1 cm
15	Hispanic	47	Multiple	15Fb	2 cm
				15Fc	4 cm
16	Hispanic	43	Multiple	16M	NR
				16Fa	2 cm
				16Fb	2 cm
17	Hispanic	39	Single	17M	NR
				17Fa	10 cm
18	Hispanic	37	Multiple	18M	NR
				18Fa	12 cm
				18Fb	3 cm
19	Hispanic	32	Multiple	19Fc	3 cm
				19Fd	1 cm
20	Hispanic	46	Multiple	20Fa	2.5 cm
				20Fc	1.5 cm
				20Fe	1 cm
				20Fh	1 cm
21	Hispanic	45	Multiple	21Fa	5 cm
				21Fb	2 cm
				21Fc	4 cm
				21Fd	3 cm

22	Hispanic	40	Multiple	22Fa	10 cm
				22Fb	1 cm
				22Fc	3 cm
				22Fd	2 cm
				22Fe	2 cm
				22Ff	2 cm
				22Fg	2 cm
23	Hispanic	40	Multiple	23M	3 cm
				23Fa	15 cm
				23Fc	3 cm
24	Hispanic	39	Multiple	24Fa	NR
				24Fb	NR
25	Hispanic	41	Multiple	25M	3 cm
				25Fa	2.5 cm
				25Fb	0.5 cm
				25Fc	1 cm
26	Hispanic	42	Multiple	26M	NR
				26Fa	15 cm
				26Fb	2 cm
27	Hispanic	50	Multiple	27M	NR
				27Fa	NR
				27Fb	NR
28	Hispanic	51	Multiple	28M	NR
				28Fa	5 cm
				28Fb	5 cm
29	Hispanic	40	Multiple	29M	3 cm
				29Fa	3 cm
				29Fb	3 cm
				29Fc	3 cm
32	Hispanic	39	Multiple	32Fa	10 cm
				32Fb	10 cm
				32Fc	8 cm
				32Fd	4 cm
34	Hispanic	35	Multiple	34Fb	NR
				34Fc	NR
				34Fe	NR
35	Hispanic	33	Single	35M	NR
				35Fa	4 cm
36	Hispanic	39	Single	36M	NR
				36Fa	NR

37	Hispanic	45	Multiple	37M	NR
				37Fa	NR
				37Fb	NR
				37Fc	NR
				37Fd	NR
39	Hispanic	37	Multiple	39M	NR
				39Fa	5 cm
				39Fb	1 cm
40	Hispanic	48	Multiple	40M	NR
				40Fb	NR
				40Fc	NR
				40Fd	NR
41	Hispanic	48	Multiple	41M	NR
				41Fa	NR
				41Fb	NR
				41Fc	NR
42	Hispanic	51	Multiple	42M	NR
				42Fa	NR
				42Fb	NR
				42Fc	NR
43	Hispanic	49	Single	43Fa	NR
44	Hispanic	49	Multiple	44M	NR
				44Fa	NR
				44Fb	NR
				44Fc	NR
47	Hispanic	41	Multiple	47M	NR
				47Fa	NR
				47Fb	NR
				47Fc	NR
50	Hispanic	61	Single	50Fa	3 cm
51	Hispanic	39	Single	51Fa	NR
86	Hispanic	37	Multiple	86M	NR
				86Fa	5 cm
				86Fb	4 cm
				86Fc	2 cm
87	Hispanic	41	Multiple	87Fc	0.5 cm
				87Fd	0.5 cm
				87Fe	2 cm
				87Ff	0.3 cm
88	Hispanic	49	Multiple	88Fa	3 cm

91	Hispanic	28	Multiple	91Fa	5 cm
				91Fb	6 cm
				91Fc	5 cm
93	Hispanic	47	Multiple	93M	NR
				93Fa	4 cm
				93Fb	3 cm
94	Hispanic	44	Multiple	94Fa	2 cm
				94Fb	1.5 cm
				94Fc	2.5 cm
95	Hispanic	41	Single	95Fa	2 cm
97	Hispanic	43	Single	97Fa	4 cm
99	Hispanic	NR	Multiple	99Fa	2 cm
				99Fb	2 cm
				99Fc	1.5 cm
102	Hispanic	34	Single	102Fa	15 cm
104	Hispanic	42	Multiple	104Fa	6 cm
				104Fb	3 cm
				104Fc	3 cm
				104Fd	2 cm
				104Fe	2 cm
				104Ff	1.5 cm
				104Fg	1 cm
				104Fh	1 cm
				104Fi	1 cm
				104Fj	0.7 cm
				104Fl	1.5 cm
				104Fn	0.8 cm
				104Fo	0.5 cm
				104Fr	1 cm
				104Fs	0.5 cm
				104Fu	0.3 cm
				104Fw	0.3 cm
				104Fx	0.3 cm
107	Hispanic	42	Multiple	107Fa	4.5 cm
110	Hispanic	42	Multiple	110Fa	1.8 cm
				110Fb	1 cm
				110Fc	0.5 cm
114	Hispanic	38	Multiple	114Fa	2 cm
				114Fb	9 cm
115	Hispanic	47	Multiple	115Fb	1.5 cm

				115Fc	1.5 cm
				115Fd	1 cm
118	Hispanic	48	Multiple	118Fa	11 cm
				118Fc	3.5 cm
				118Fd	3.8 cm
				118Fe	2.0 cm
119	Hispanic	35	Multiple	119Fa	8 cm
				119Fc	2 cm
120	Hispanic	41	Single	120Fa	0.5 cm
121	Hispanic	60	Multiple	121Fa	4 cm
				121Fb	2.5 cm
				121Fc	0.3 cm
				121Fd	0.5 cm
122	Hispanic	45	Multiple	122Fc	0.3 cm
				122Fd	0.3 cm
125	Hispanic	48	Multiple	125Fa	3.5 cm
				125Fb	1.2 cm
				125Fd	0.7 cm
				125Fe	1.5 cm
				125Ff	1 cm
				125Fg	0.8 cm
				125Fi	0.5 cm
				125Fj	0.3 cm
126	Hispanic	45	Multiple	126Fa	0.8 cm
				126Fb	0.5 cm
				126Fc	0.7 cm
				126Fe	0.6 cm
				126Ff	0.6 cm
				126Fg	0.3 cm
				126Fh	0.7 cm

M: Myometrium

F: Uterine Fibroid

NR: Not Reported

Supplemental Table S2

Table S2. Summary of clinicopathological data for non-Hispanic UF patients

Individual	Ethnicity	Age	Solitary/Multiple Fibroids	Uterine Fibroids	Diameter (cm)
6	Caucasian	33	Single	6a	4 cm
9	African American	50	Multiple	9Fc	3 cm
13	African American	57	Multiple	13Fa	NR
				13Fb	NR
30	Caucasian	36	Multiple	30M	NR
				30Fa	8 cm
				30Fb	5 cm
				30Fc	3 cm
38	Caucasian	47	Multiple	38M	3 cm
				38Fa	5 cm
				38Fb	5 cm
				38Fc	3 cm
45	Chinese	34	Single	45Fa	12 cm
46	African American	30	Multiple	46Fa	3 cm
				46Fb	3 cm
				46Fc	2 cm
				46Fd	2 cm
				46Fe	1.5 cm
48	Caucasian	43	Multiple	48Fa	1 cm
				48Fb	1 cm
49	African American	32	Single	49Fa	4 cm
52	African American	39	Multiple	52Fa	NR
				52Fb	NR
53	African American	54	Multiple	53M	4 cm
				53Fb	7 cm
				53Fc	5 cm
54	Caucasian	36	Multiple	54M	1 cm
				54Fa	10 cm
				54Fc	2 cm
55	Caucasian	44	Multiple	55M	NR
				55Fa	6 cm
				55Fb	2 cm

				55Fc	1 cm
65	African American	50		65M	NR
85	African American	37	Multiple	85Fa	NR
				85Fb	NR
				85Fc	NR
89	Caucasian	44	Multiple	89Fa	18 cm
				89Fb	5 cm
				89Fc	2 cm
90	Caucasian	37	Multiple	90M	NR
				90Fb	2 cm
				90Fc	1 cm
				90Fd	0.5 cm
				90Fe	0.5 cm
				90Fi	1 cm
				90Fl	1 cm
				90Fn	0.4 cm
				90Fp	0.2 cm
96	African American	39	Multiple	96Fa	2.5 cm
				96Fb	2.5 cm
				96Fc	2 cm
				96Fd	1.5 cm
				96Fe	0.5 cm
				96Fg	0.5 cm
98	Iranian	47	Single	98Fa	1.5 cm

M: Myometrium

F: Uterine Fibroid

NR: Not Reported

Supplemental Table S3

Table S3. Summary of MED12 mutation status in Hispanic UF patients

Individual	Myo/Fib	MED12 Status
7	7b	wt
10	10Fa	c.130G>T, p.G44C
10	10Fb	c.107T>G, p.L36R
10	10Fc	c.130G>A, p.G44S
10	10Fd	c.131G>A, p.G44D
10	10Fe	c.130G>A, p.G44S
10	10Ff	c.130G>A, p.G44S
10	10Fg	c.131G>A, p.G44D
10	10Fh	c.107T>G, p.L36R
10	10Fi	c.131G>A, p.G44D
10	10Fj	c.130G>A, p.G44S
10	10Fk	c.131G>A, p.G44D
10	10Fl	c.107T>G, p.L36R
11	11Fa	c.131G>T, p.G44V
11	11Fb	c.131G>A, p.G44D
11	11Fc	c.204A>G, p.K68E
11	11Fd	c.130G>A, p.G44S
12	12F	c.130G>A, p.G44S
MRKH	MRKH-M	wt
MRKH	MRKH-F	c.131G>A, p.G44D
14	14Fa	c.131G>A, p.G44D
15	15Fb	c.117_131del15, p.L39P_G44del
15	15Fc	c.130G>A, p.G44S
16	16M	wt
16	16Fa	wt
16	16Fb	wt
17	17M	wt
17	17Fa	wt
18	18M	wt
18	18Fa	wt
18	18Fb	wt
19	19Fc	c.131G>A, p.G44D
19	19Fd	wt
20	20Fa	c.131G>A, p.G44D
20	20Fc	c.131G>A, p.G44D
20	20Fe	c.130G>C, p.G44R
20	20Fh	c.131G>A, p.G44D
21	21Fa	wt
21	21Fb	wt
21	21Fc	wt
21	21Fd	wt
22	22Fa	c.107T>G, p.L36R
22	22Fb	c.131G>C, p.G44A
22	22Fc	c.130G>T, p.G44C
22	22Fd	c.131G>T, p.G44V
22	22Fe	c.131G>T, p.G44V
22	22Ff	c.100-9_132del42insGG, p.D34_G44del
22	22Fg	c.130G>C, p.G44R
23	23M	wt
23	23Fa	wt
23	23Fc	wt
24	24Fa	wt
24	24Fb	wt
25	25M	wt
25	25Fa	wt
25	25Fb	wt
25	25Fc	wt
26	26M	wt
26	26Fa	wt
26	26Fb	wt
27	27M	wt
27	27Fa	c.131G>T, p.G44V
27	27Fb	c.131G>A, p.G44D
28	28M	wt

Individual	Myo/Fib	MED12 Status
28	28Fa	wt
28	28Fb	c.100-2_138del41, p.D34_N46del
29	29M	wt
29	29Fa	c.131G>A, p.G44D
29	29Fb	wt
29	29Fc	c.131G>A, p.G44D
32	32Fa	wt
32	32Fb	wt
32	32Fc	wt
32	32Fd	wt
34	34Fb	c.139_153del15, p.N47_V51del
34	34Fc	wt
34	34Fe	wt
35	35M	wt
35	35Fa	wt
36	36M	wt
36	36Fa	c.131G>A, p.G44D
37	37M	wt
37	37Fa	wt
37	37Fb	wt
37	37Fc	wt
37	37Fd	wt
39	39M	wt
39	39Fa	wt
39	39Fb	wt
40	40M	wt
40	40Fb	wt
40	40Fc	wt
40	40Fd	wt
41	41M	wt
41	41Fa	wt
41	41Fb	wt
41	41Fc	c.131G>C, p.G44A
42	42M	wt
42	42Fa	wt
42	42Fb	wt
42	42Fc	c.131G>A, p.G44D
43	43Fa	c.130G>A, p.G44S
44	44M	wt

Individual	Myo/Fib	MED12 Status
44	44Fa	wt
44	44Fb	wt
44	44Fc	wt
47	47M	wt
47	47Fa	wt
47	47Fb	wt
47	47Fc	c.130G>A, p.G44S
50	50Fa	wt
51	51Fa	c.131G>A, p.G44D
86	86M	wt
86	86Fa	wt
86	86Fb	wt
86	86Fc	wt
87	87Fc	wt
87	87Fd	c.131G>A, p.G44D
87	87Fe	wt
87	87Ff	c.131G>T, p.G44V
88	88Fa	c.131G>A, p.G44D
91	91Fa	wt
91	91Fb	wt
91	91Fc	wt
93	93M	wt
93	93Fa	c.130G>T, p.G44C
93	93Fb	wt
94	94Fa	wt
94	94Fb	wt
94	94Fc	wt
95	95Fa	wt
97	97Fa	wt
99	99Fa	c.131G>T, p.G44V
99	99Fb	c.131G>T, p.G44V
99	99Fc	c.130G>A, p.G44S
102	102Fa	c.131G>A, p.G44D
104	104Fa	wt
104	104Fb	c.130G>C, p.G44R
104	104Fc	c.130G>C, p.G44R
104	104Fd	wt
104	104Fe	c.131G>C, p.G44A
104	104Ff	c.130G>C, p.G44R

Individual	Myo/Fib	MED12 Status
104	104Fg	wt
104	104Fh	c.130G>A, p.G44S
104	104Fi	c.130G>A, p.G44S
104	104Fj	c.130G>A, p.G44S
104	104Fl	c.130G>T, p.G44C
104	104Fn	c.107T>G, p.L36R
104	104Fo	c.130G>T, p.G44C
104	104Fr	c.130G>A, p.G44S
104	104Fs	c.130G>A, p.G44S
104	104Fu	c.130G>T, p.G44C
104	104Fw	c.130G>C, p.G44R
104	104Fx	c.107T>G, p.L36R
107	107Fa	c.107T>G, p.L36R
110	110Fa	wt
110	110Fb	c.131G>T, p.G44V
110	110Fc	wt
114	114Fa	wt
114	114Fb	wt
115	115Fb	wt
115	115Fc	c.107T>G, p.L36R
115	115Fd	c.131G>A, p.G44D
118	118Fa	wt
118	118Fc	c.131G>A, p.G44D
118	118Fd	c.130G>A, p.G44S
118	118Fe	c.131G>A, p.G44D
119	119Fa	wt
119	119Fc	wt
120	120Fa	wt
121	121Fa	c.130G>A, p.G44S
121	121Fb	c.131G>A, p.G44D
121	121Fc	c.131G>A, p.G44D
121	121Fd	c.130G>A, p.G44S
122	122Fc	wt
122	122Fd	c.130G>T, p.G44C
125	125Fa	c.131G>T, p.G44V
125	125Fb	c.131G>T, p.G44V
125	125Fd	c.131G>A, p.G44D
125	125Fe	c.130G>A, p.G44S
125	125Ff	c.131G>A, p.G44D
125	125Fg	c.131G>A, p.G44D
125	125Fi	wt
125	125Fj	wt
126	126Fa	c.130G>A, p.G44S
126	126Fb	c.130G>A, p.G44S
126	126Fc	c.131G>A, p.G44D
126	126Fe	c.131G>T, p.G44V
126	126Ff	wt
126	126Fg	c.131G>A, p.G44D
126	126Fh	c.131G>A, p.G44D

Myo: Myometrium

Fib: Uterine Fibroid

wt: wild type

Supplemental Table S4

Table S4. Summary of MED12 mutation status in non-Hispanic UF patients

Individual	Myo/Fib	MED12 Status
6	6a	wt
9	9Fc	wt
13	13Fa	c.131G>C, p.G44A
13	13Fb	c.131G>T, p.G44V
30	30M	wt
30	30Fa	c.130G>T, p.G44C
30	30Fb	c.107T>G, p.L36R
30	30Fc	c.131G>A, p.G44D
38	38M	wt
38	38Fa	wt
38	38Fb	wt
38	38Fc	wt
45	45Fa	wt
46	46Fa	wt
46	46Fb	c.130G>C, p.G44R
46	46Fc	wt
46	46Fd	wt
46	46Fe	c.130G>C, p.G44R
48	48Fa	wt
48	48Fb	wt
49	49Fa	wt
52	52Fa	wt
52	52Fb	wt
53	53M	wt
53	53Fb	c.124_153del30, p.K42_V51del
53	53Fc	c.131G>T, p.G44V
54	54M	wt
54	54Fa	c.131G>T, p.G44V
54	54Fc	c.130G>A, p.G44S
55	55M	wt
55	55Fa	wt
55	55Fb	wt
55	55Fc	c.131G>T, p.G44V
65	65M	wt
85	85Fa	c.131G>A, p.G44D
85	85Fb	c.131G>A, p.G44D
85	85Fc	wt
89	89Fa	wt
89	89Fb	c.131G>A, p.G44D
89	89Fc	wt
90	90M	wt
90	90Fb	c.131G>A, p.G44D
90	90Fc	c.131G>T, p.G44V
90	90Fd	c.130G>A, p.G44S
90	90Fe	c.131G>T, p.G44V
90	90Fi	c.131G>A, p.G44D
90	90Fl	c.131G>A, p.G44D
90	90Fn	c.131G>C, p.G44A
90	90Fp	c.131G>A, p.G44D
96	96Fa	c.130G>A, p.G44S
96	96Fb	c.107T>G, p.L36R
96	96Fc	c.130G>A, p.G44S
96	96Fd	wt
96	96Fe	c.100_144del45, p.D34_Q48del
96	96Fg	c.131G>T, p.G44V
98	98Fa	c.131G>A, p.G44D

Myo: Myometrium

Fib: Uterine Fibroid

wt: wild type